# Response to Questions from the Institute of Medicine

6. *What endpoints would you recommend for assessing harm reduction through use of reduced risk products or by decreasing consumption of existing products via use of nicotine replacement therapies or other pharmaceutical products?*

(5)

For a complete assessment of the potential for harm reduction, the harmful effect, its dose-response relationship, and the exposure in the respective situation need to be identified. Since exposure assessment will be dealt with elsewhere (question 7), this response will solely deal with surrogate endpoints to assess the effects and their dose-response relationship in surrogate models. It should be clear that the extent of testing for a claim on harm reduction exceeds what we have recently considered to be required in terms of acceptability testing for market introduction of changes in cigarette design or ingredient additions.

We consider four tiers of endpoints to be useful to assess the potential for harm reduction:

(1) chemical analysis of cigarette smoke,
(2) experimental toxicology (pre-clinical endpoints),
(3) clinical tests, and
(4) epidemiology.

With the exception of the first tier, these should be applicable to both types of harm reduction strategies, *i.e.*, for reduced risk products as well as for decreased consumption of existing products in combination with pharmaceutical products. It should be mentioned that to our knowledge there are no precedents for the assessment for harm reduction in the case of using pharmaceutical products, such as nicotine replacement therapies, in combination with a reduced consumption of existing products. As a preliminary statement, based on Dr. N. Benowitz's ~~lecture~~ presentation to this committee on nicotine pharmacology and toxicology, toxic effects of nicotine by itself seem to be very limited and are no more than those of cigarette smoking (see also Benowitz and Gourlay, 1997).

The assays used to assess the potential for harm reduction should be related to the mechanisms of the smoking-related diseases. They need to be validated, *e.g.*, based on the recommendations of the International Congress of Harmonization, but at least to the degree of reproducibility within and between laboratories. They should provide quantitative data in order to allow dose-response evaluations.



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(1) Chemical Analysis of Cigarette Smoke

We suggested analyzing a list of smoke constituents that is based on the 1993 recommendation of the Consumer Product Safety Commission and extended towards known smoke constituents that have been classified by IARC as class A, 2A, or 2B carcinogens (Voncken et al., 1998). This extension was deemed useful based on the current lack of an established inhalation carcinogenicity model for cigarette smoke (*vide infra*). Our current list comprises more than 50 smoke constituents. Similar list have been suggested by others (Vorhees et al., RJR).

With this number of endpoints, there will be increases in yields of certain constituents in parallel to decreases in others. The results of these divergent changes in the chemical composition need to be consolidated with a sound toxicological procedure, since, *e.g.*, new products that may show advantages in many endpoints may not be sacrificed because of an unfavorable outcome for a few less relevant endpoints. We are currently working on such a procedure.

(2) Experimental Toxicology (Pre-Clinical Endpoints)

Most currently used experimental endpoints in routine cigarette smoke toxicology are directed towards chemical carcinogenesis.

We have adopted the recommendations of the International Congress of Harmonization (ICH) to assess the genotoxicity of cigarette smoke. This recommendation includes a bacterial mutagenicity assay, a mammalian cell mutagenicity or clastogenicity assay, and an *in vivo* genotoxicity assay. We have routinely been using the *Salmonella typhimurium* reverse mutation assay with 5 tester strains in the absence and presence of a pro-mutagen activation system . For the assessment of harm reduction, however, only those assay variants responding to cigarette smoke can be used, i.e., mainly strains TA98 and TA100 in the absence and presence of the activation system (Roemer et al., 1998). As a mammalian test system for *in vitro* genotoxicity, we use the mouse lymphoma assay, which covers both mutagenic and clastogenic endpoints of genotoxicity but in our hands shows less discriminatory power when comparing different cigarette types than the bacterial assay. To our knowledge, there is no *in vivo* genotoxicity assay available that reproducibly responds to cigarette smoke. While a number of publications have been published on the activity of cigarette smoke to induce bone marrow micronuclei in mice or rats (Balansky et al., 1999), we as well as others (Lee et al., 1990) were not able to reproduce these findings, even under extreme testing conditions. Other *in vivo* assays have been suggested but not routinely and reproducibly applied to cigarette smoke, *e.g.*, cytogenetic changes in alveolar macrophage (Rithideh et al., 19xx; Balansky et al., 1999). There are potential other assays that need to be



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evaluated for their use with cigarette smoke, *e.g.*, the Comet assay for target cells or surrogate cells (peripheral lympocytes) *ex vivo* or transgenic rodents engineered as *ex vivo* genotoxicity models.

Because of the regressing relative risk for lung cancer after smoking cessation, we consider assays important that are considered to assess the promotion phase of carcinogenesis. Exposure of rodents to cigarette smoke as well as other irritant inhalants generates regenerative hyperplastic responses in the epithelia lining the respiratory tract, which are reversible upon cessation of exposure. These as well as the metaplastic changes observed in subchronic inhalation studies can be considered surrogate assays for the promoting potency of cigarette smoke. As an *in vitro* model, cytotoxicity assays for both the total particulate matter and the gas phase of cigarette smoke have been routinely used. Other *in vitro* assays that were recommended to assess the promoting activity of cigarette smoke are 1-stage or 2-stage transformation assays. In our experience, these assays respond to cigarette smoke, however with a poor dose-response characteristic and a poor discriminatory power (Schlage et al., 1999), and thus are not considered useful to accurately assess the potential for harm reduction.

Carcinogenicity studies using inhalation exposure of rodents to cigarette smoke have not been successful in generating lung tumors (Coggins et al., 1998). Recent efforts to improve this approach have aimed at optimizing the dose (Lovelace Respiratory Research Institute (LRRI), 19xx), the sensitivity of the detection of tumors (Philip Morris study: Stinn et al., 1999; Friedrichs et al., 2000) or using a model that is very susceptible to lung tumor formation but has previously had insufficient regulatory reputation (Witschi et al., 1998). While the other studies have not yet been reported in detail, the Witschi model has some mechanistic issues that need to be resolved before it can be used for routine testing for harm reduction. As a substitute model to produce epithelial tumors from a cigarette smoke fraction, *i.e.*, condensate, mouse skin painting has been performed (ref.). Since this model does not administer the whole smoke by inhalation and studies tumors at a non-target site, we consider it as useful to answer specific mechanistic questions but not sufficiently relevant for routine testing.

Experimental surrogate assays that would be specific for non-carcinogenic endpoints have not routinely been used for assessing cigarette smoke. Some rough information could be obtained by evaluating the organs specified in the OECD guideline no. 4xx in the course of a subchronic inhalation study. For example, representative sections from the heart and aorta as well as from urogenital organs have been evaluated beyond the organs and tissues of the respiratory tract (ref.). Only some changes in organ weight (liver, heart, adrenals, thymus, depending on the



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exposure schedule) with no histological correlates have been observed in studies using rats, with no indications of, *e.g.*, emphysematous or atherosclerotic changes. A large number of assays has been used as investigative tools but not for routine testing of cigarette prototypes. These assays thus currently lack the validation that would be required for their application to test for potential harm reduction.

For cardiovascular disease endpoints, atherosclerosis, vascular tone changes, and coagulopathy are the major mechanistic effects involved. A number of *in vitro*, *ex vivo*, and *in vivo* tests have been used to mechanistically study the effects of cigarette smoke and might be applicable upon further validation for routine testing. Here we can only provide some examples that might be considered for further investigation and potentially validation, *i.e.*, the *in vitro* oxidant challenge of plasma lipoproteins with cigarette smoke (Eiserich et al., 1995), the *ex vivo* vasodilatory effect of acetylcholine in isolated blood vessels (ref.), the atherosclerosis in ApoE-deficient mice after subchronic exposure (Breslow, 1996; Gairola and Daugherty, 1999), and the leukocyte aggregation and adhesion to endothelium in acutely exposed hamsters (Lehr et al., 1994). These endpoints are considered to be mechanistically related to the effects seen in human smokers.

The database for experimental reprotoxicological endpoints is relatively small. We are not aware of any test on cigarette smoke that is based on international guidelines for reproductive toxicology studies. An extended literature search is currently being performed to determine the smoke-related effects in humans and their potential experimental surrogates.

Chronic obstructive pulmonary diseases are characterized by pulmonary inflammation, the obstruction of airways by mucus and morphological changes, and emphysema. Surrogate endpoints are being established to assess these effects both *in vitro* and *in vivo*. The rat responds to subchronic cigarette smoke inhalation with a bronchoalveolar accumulation of polymorphonuclear leukocytes and macrophages which is similar to the disease symptoms in human smokers (Kindt et al., unpublished; ref.?). A chronic mouse emphysema model has been suggested by March et al. (1999) and needs further evaluation. As an *in vitro* surrogate, the emphysematous disturbance of the protease/anti-protease balance in the lungs can be assessed (Pryor et al., 1990). This endpoint might also be assessable *ex vivo*.

(3) Clinical Tests

For the purpose of supporting potential claims on harm reduction, we consider clinical tests to some extent as necessary. Since Philip Morris has not made such claims, we do not have [redacted] experience in assessing potential endpoints and study design as of to date.



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These tests should be performed based on internationally accepted guidelines (e.g., Helsinki convention, International Congress of Harmonization, Good Clinical Practice) and could parallel sequential test market approaches for the subjective testing of new products. For these tests, solid exposure assessment and control of compliance with the study design is an absolute pre-requirement (see question 7).

In general, one might differentiate between acute (minutes to days) and sub-acute (weeks to months of use of the new product) tests. Also, the degree of invasiveness of the assays needs to be considered. The following list of potential assays should be considered explorative and by no means conclusive, and needs to be further evaluated based on literature reviews and discussions with experts.

A number of acute markers for carcinogenicity have been suggested. Urinary mutagenicity has been considered a measure of exposure and activation/deactivation of mutagens and pro-mutagens. Acute genotoxic effects have been suggested to be assessed by cytogenetic changes in peripheral lymphocytes (chromosomal aberration, sister chromatid exchange, single cell electrophoresis for DNA breaks). Likewise, these genotoxic effects could be assessed using free or exfoliated cells from the bronchoalveolar space, obtained either by lavage or induced sputum samples. However, while bronchoalveolar lavage might be considered relatively invasive, induced sputum analysis is still too poorly investigated. The latter cells could also be used for assays on loss of heterozygosity or mutations in proto-oncogenes and tumor suppressor genes. We are not aware of any clinical endpoint that would allow the determination of morphological changes associated with reduced promotion of lung tumorigenicity except biopsy. Embryonic protein markers would also need valdiation.

For cardiovascular effects, degradation products of thromboxane and prostacycline could be determined in urine after acute exposure. Also, platelet aggregability and monocyte/endothelial adhesion could be assessed. The ratio of high density and low density lipoproteins seems to react fairly quickly to smoke exposure. Flow dynamic changes may also be assessed.

We are currently not aware of clinical markers for reproductive toxicology that would be applicable in the context of assessing the potential for harm reduction.

In addition to respiratory changes, chronic bronchitis may be assessed by analyzing cells or cytokines in bronchoalveolar lavage or induced sputum, with the restrictions as mentioned above. It remains to be determined whether an impaired protease/anti-protease balance can also be analyzed in these media.



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While no geulatorily accepted and validated assays in the sense of experimental toxicology are currently available for the clinical testing for the claim for reduced harm tobacco products, reasonable approaches to support such a claim nowadays would need a broad scientific discussion of endpoints used and data obtained in every single case in order to find consensus.

(4) Epidemiology

We consider epidemiological studies necessary to follow up on newly introduced products that claim reduced harm. Data from these studies will provide the final evidence for harm reduction and can be used to further validate previous pre-clinical and clinical studies. Epidemiological studies are also needed to make sure that, while one adverse effect decreases as intended, no other adverse effects increase or newly arise. Finally, these epidemiological studies need to be accompanied by repeated exposure studies to determine possible changes in the smoking behavior.

Summary

We consider four tiers of endpoints to be useful to assess the potential for harm reduction: chemical smoke analysis, pre-clinical and clinical tests, and epidemiology. These tiers go beyond what currently is considered necessary for market acceptability testing. Chemical smoke analysis and pre-clinical testing should be performed before any test marketing. Clinical test should parallel sequential steps in test marketing. Epidemiology is only possible after full market introduction. Endpoints for chemical smoke analysis and pre-clinical endpoints in the context of carcinogenicity are available and being routinely used, while pre-clinical endpoints for non-neoplastic diseases need to be further validated. For clinical tests, there is no common regarding the choice of endpoints, and there are insufficient data on reproducibility or other types of validation for probably most of these assays. For the time being, reasonable approaches need to be scientifically discussed to gain consensus among the stakeholders. Epidemiological data are needed on the long run to ascertain the intended harm reduction and to further validate pre-clinical and clinical testing strategies.

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